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THE INFLUENCE OF A HIGH LEVEL OF CORN OIL ON RAT SERUM LIPOPROTEINS[1,2]

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Summary

Although the stated requirement for linoleic acid in humans is less than 2% of the dietary calories, recently there has been considerable emphasis on the necessity to substitute dietary polyunsaturates for saturates in order to reduce serum cholesterol levels. In this study we have sought to determine the nutritional consequences of feeding a very high level of linoleate to rats. Three groups of thirty adult animals each were fed a semipurified diet consisting by weight of casein 17%; mineral mixture 5.5%; vitamin mixture in glucose 2.2%; cellulose fiber 3.0%; and corn oil 0% (group A), 10% (group B) or 40% (group C), which was provided at the expense of glucose. At the end of four weeks on the diets, blood was obtained in the fasting state from 16 rats in each group. The serum was ultracentrifugally fractionated into six classes of lipoproteins and analyzed for lipid composition and protein content. Disc gel electrophoresis using lipid and protein stains established that the various lipoprotein subclasses were reasonably free of adjacent density fractions. Although the total serum cholesterol levels were practically the same in the three groups, the cholesterol moiety of the major low density lipoproteins, LDL_2 (d 1.019—1.050), but not of very low density lipoproteins, VLDL (d 1.006) or low density lipoproteins, LDL_1 (d 1.006—1.019), was substantially and very significantly increased in rats fed the high level of corn oil as compared to the other groups. The concen-

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[2] A companion study describing the progressive alterations in serum and liver lipids of rats fed different levels of corn oil has been reported elsewhere (ref. [21]).
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tration of the very low density lipoproteins was significantly lower in group C than in the groups A and B. The LDL_2 concentration but not that of LDL_1 was significantly greater in group C as compared to group A. The cholesterol/total lipid ratio was significantly greater in both LDL_2 and LDL_1 but not in VLDL of group C as compared with group A. The serum high density lipoproteins were relatively less influenced by the ingestion of an excessive level of corn oil at this time period. The serum lipoprotein levels as well as their lipid composition were generally similar in groups A and B and suggested that a moderate level (5%) of dietary linoleate did not cause any untoward changes in rats. On the basis of current information on the metabolism of lipoproteins, it has been proposed that the increase in rat serum LDL_2 of group C reflects the status of the liver and that a large portion of the cholesterol moiety of LDL_2 is perhaps derived from the liver while the protein and phospholipid portions may represent remnants of VLDL catabolism. In view of the magnitude of the changes observed in LDL_2-cholesterol as well as in the liver cholesterol and triglycerides due to the ingestion of a 40% corn oil diet in a usually resistant species, namely the rat, further work along these lines with other species including human and nonhuman primates merits our attention.

Key words: *Cholesterol — Lipoprotein metabolism — Phospholipid — Polyacrylamide gel electrophoresis — Rat serum albumin — Triglyceride — Unsaturated fat — Very low to low density lipoprotein transformation*

Introduction

While the rat is widely used in many nutritional and biochemical investigations concerning the role of dietary fat, there is insufficient information on the various subclasses of rat serum lipoproteins as a result of nutritional manipulations. In spite of recent information concerning the apolipoprotein peptides, lipid and peptide exchanges, and other structural aspects of lipoproteins in normal animals and humans [1—5] it is still necessary to know how dietary factors alter lipoprotein profiles and may predispose to the deposition of lipids in the liver, adipose tissue, vasculature and other sites. In earlier investigations, it was shown that the rat serum electrophoretic pattern was uniquely different from that of other species [6,7], that it was easily possible to separate high density lipoprotein HDL_1 [8] from the normal low density lipoprotein fraction, and that the high density lipoprotein HDL_2 [9] was the predominant high density lipoprotein in male Holtzman rats.

The introduction of special formula foods such as corn oil-containing margarines and egg products as well as of beef and butter high in polyunsaturates [10] has stimulated considerable interest in the beneficial properties of dietary linoleate. Additional impetus to the use of increased levels of dietary linoleate was provided by the recent statement of the NAS-NRC and AMA Committee on Diet and Coronary Heart Disease [11]. However, the optimum level of fat

to be used or the precise degree of substitution of polyunsaturated for saturated fat is still undefinable on the basis of present information. For many military food items which are stored under demanding conditions for extended periods, high levels of polyunsaturates can lead to oxidative changes and result in off-flavors, poor acceptability and reduced nutrient availability. Several studies, especially those by Holman [12] have well established the need for a limited amount (1 to 2% of the total calories) of dietary linoleate. However, there is very little information concerning the nutritional consequences of feeding excessive amounts of linoleate to either animals or humans. The effect of dietary substitution of linoleate for saturates as well as supplementation of cholesterol have been extensively investigated in humans by following changes in serum cholesterol levels [13,14]. In the rat, it was previously shown that a dietary supplementation of cholesterol produced drastic but compensatory changes in serum low density and high density lipoproteins and, thus, resulted in only minimal changes in serum cholesterol levels [15]. In an attempt to determine the possible consequences of high intakes of polyunsaturates, we have examined the concentration and lipid composition of six density classes of serum lipoproteins of rats fed widely divergent levels of corn oil. Data on weight gain, food intake and organ weights are also reported.

Experimental

Animals and diets

Ninety male Holtzman rats (av. wt. 0.3 kg) were divided into 3 equal groups and were fed a semipurified diet containing by weight: casein 17%; vitamin mixture (Nutritional Biochemicals, Cleveland, Ohio) 2.2%; minerals, Rogers and Harper (Nutritional Biochemicals, Cleveland, Ohio) 5%, plus an additional mineral mixture to bring it to NRC recommendations [16]; cellulose fiber 3%; corn oil (approximately 50% linoleic acid) 0% (group A), 10% (group B) or 40% (group C) provided at the expense of glucose, which was the sole source of carbohydrate. The animals were housed in individual cages and were given food and water ad libitum.

Tissue samples

After 4 weeks on the diet, 16 rats from each group were fasted 16 hr before collection of blood from the abdominal aorta. For the ultracentrifugal isolation of serum lipoproteins, 4 pools of sera were obtained for each group using 4 rat sera per each pool.

Ultracentrifugal isolations

Preparative centrifugation was initiated the day of the collection of rat blood and a total of 6 lipoprotein fractions and 2 serum protein fractions were isolated [15,17] in succession at 114,480 $\times g$ in a Beckman Spinco Model L ultracentrifuge using a 40.3 rotor at 10° C as follows: Very low density lipoproteins (VLDL, $d < 1.006$) were isolated by centrifuging for 20 hr; low density lipoproteins, LDL_1 ($1.006 < d < 1.019$) for 20 hr; low density lipoproteins, LDL_2 ($1.019 < d < 1.050$) for 20 hr; high density lipoproteins, HDL_1 ($1.050 < d < 1.063$) for 20 hr; high density lipoproteins, HDL_2 ($1.063 < d < 1.125$) for 22 hr; high

density lipoproteins, HDL_3 ($1.125 < d < 1.21$) for 23 hr. Since the HDL_3 fraction was heavily contaminated with albumin, it was recentrifuged at d 1.21 under the same conditions. The top fraction from this centrifugation was called HDL_3-P (HDL_3, purified) fraction and the bottom fraction from this step was referred to as the HDL_3-impurities. In all cases, the top layer (lipoprotein fraction) removed from each centrifuge tube (usually 6.0 ml added) was 1.2 ml. All fractions were exhaustively dialyzed against physiological saline containing 0.1% merthiolate and 0.005% versene.

Polyacrylamide disc gel electrophoresis

Electrophoresis of Sudan black B-prestained serum lipoproteins was carried out in 3.75% polyacrylamide gel by the procedure described earlier [6]. The isolated serum lipoproteins were also subjected to analytical disc electrophoresis using a protein stain according to the procedure of Narayan *et al.* [18]. The distance of penetration of the tracking dye was generally set at 2.8 cm when the Amido black stain was used. With the Sudan black prestain, because of the absence of tracking dye or a visible lead band (albumin component) no precise measurements were possible. However, in most cases, the extent of penetration would be comparable to that usually obtained with the prestained serum patterns [6] (approximately 1.2 cm).

Chemical analysis

The procedure for lipid extraction and the assay of protein, total lipid, cholesterol, and phospholipid were the same as before [15]. In some cases, especially VLDL, protein determinations were modified as recommended by Kruski and Narayan [19]. The total lipids were determined independently using a dichromate oxidation procedure [15] and the values reported here for cholesterol are expressed as unesterified cholesterol. Triglycerides were analysed by the method of Van Handel and Zilversmit [20].

Results

The data show that in rats given a high level of dietary linoleate (40% corn oil) for 4 weeks there was a pronounced decrease in serum VLDL, a small decrement in serum HDL_2-phospholipids and a large increment in serum LDL_2 as compared to rats in group A. However, no extraordinary changes were observed in rat body and tissue weights in the three groups. Between group A and B the lipoprotein values were largely unaffected and appeared to reflect the status of the liver lipids [21] in these groups.

Concentration, composition and disc gel electrophoretic patterns of rat serum lipoproteins as influenced by the dietary level of corn oil

VLDL ($d < 1.006$)

The concentration of serum VLDL was significantly decreased in group C as compared to both groups A and B (Table 1). While the protein content was about the same in the three groups, the lipid moiety was considerably lower in group C as compared with the other groups. All lipid classes progressively de-

TABLE 1

THE INFLUENCE OF DIETARY CORN OIL ON RAT SERUM VLDL[a]

Group No. (% fat by wt.)	Total lipid	Protein	Lipoprotein	Cholesterol	Phospholipids	Triglycerides
	(mg/100 ml serum)					
A (0% corn oil)	65.6 ± 18	4.80 ± 0.67	70.4 ± 18	5.65 ± 1.1	10.6 ± 2.4	38.3 ± 7.3
B (10% corn oil)	50.6 ± 9.7	4.65 ± 0.66	55.3 ± 10	4.75 ± 0.97	9.20 ± 1.8	26.6 ± 7.5
C (40% corn oil)	36.5 ± 5.3	4.05 ± 0.38	40.8 ± 5.2	3.95 ± 0.19	6.40 ± 0.91	15.8 ± 5.2
	($P < 0.025$)[b] ($P < 0.05$)[c]		($P < 0.025$)[b] ($P < 0.05$)[c]		($P < 0.025$)[b] ($P < 0.05$)[c]	($P < 0.005$)[b]

[a] All values are mean ± SD of 4 serum pools.
[b] Compared to group A.
[c] Compared to group B.

creased in concentration from group A to group B to group C, but only the phospholipid and triglycerides were significantly lower in group C as compared to group A. The percentage of triglycerides in the total lipids of VLDL was considerably lower, but that of cholesterol and phospholipids was not significantly altered in group C as compared with the other groups, particularly group A. The large decrease in serum VLDL concentration in group C is consistent with our findings on the decrease in serum triglycerides [21] in group C as compared with groups A and B.

In all VLDL fractions, only one main gel component was observed using the lipophilic dye (Fig. 1 a, b, c). Sudan black B-prestain has the disadvantage that its stain intensity is not directly proportional to lipoprotein concentration and also that the intensity of the spacer bands may be masked by excess precipitated dye [22]. With Amido black stain, the presence of spacer components (very large VLDL macromolecules) was confirmed in all groups (Fig. 1 d, e, f). With the exception of a trace albumin component, there was only one main gel component in all groups. Thus, it was clear that these VLDL fractions were uncontaminated with LDL and HDL.

LDL_1 ($1.006 < d < 1.019$)

Relatively small changes were observed in this lipoprotein fraction as a result of the dietary treatments (Table 2). However, in group C the cholesterol, but not phospholipids, expressed as percent of the total lipids in LDL_1, was significantly higher than in the other groups. With the lipid prestain, a very faint main gel band was observed in all groups (Fig. 1 g, h, i). Using protein stain, a better definition of the bands in the main gel was obtained. A non-migrating band (juncture of spacer and main gels) and one to two fainter components were noticeable in addition to the albumin band (Fig. 1 j, k, l). The spacer gels were almost clear and suggested that the bands observed with Sudan black B-prestain in this fraction may be artifacts caused by excess dye (Fig. 1 g, h, i).

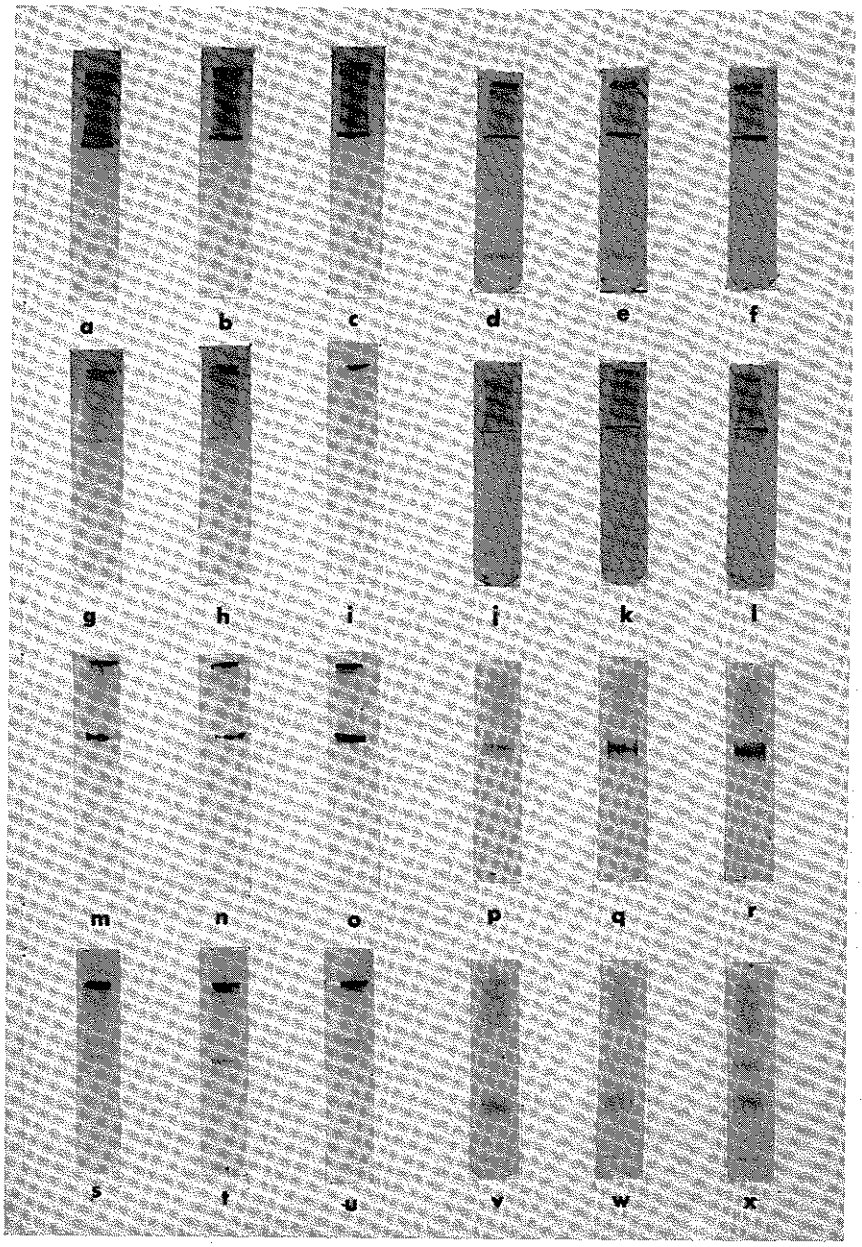

Fig. 1. Representative disc gel electrophoretic patterns of isolated lipoproteins from 3 groups. *a—f*, VLDL; *g—l*, LDL_1; *m—r*, LDL_2; *s—x*, HDL_1. *a, b, c, g, h, i, m, n, o, s, t, u*, Sudan black B-prestain, the rest Amido black stain. *a, d, g, j, m, p, s, v*, group A; *b, e, h, k, n, q, t, w*, group B; *c, f, i, l, o, r, u, x*, group C.

LDL_2 ($1.019 < d < 1.050$)

This lipoprotein is a major carrier of cholesterol in rat serum and the concentration of serum LDL_2 was observed to be progressively increased with increasing level of corn oil in the diet (Table 3). However, statistical significance could be

TABLE 2

THE INFLUENCE OF DIETARY CORN OIL ON RAT SERUM LDL_1 [a]

Group No. (% fat by wt.)	Total lipid	Protein	Lipoprotein	Cholesterol	Phospholipids
	(mg/100 ml serum)				
A (0% corn oil)	13.0 ± 2.7	3.73 ± 0.37	16.7 ± 3.0	1.01 ± 0.36	1.92 ± 0.39
B (10% corn oil)	19.4 ± 6.7	3.73 ± 0.21	23.1 ± 7.0	1.17 ± 0.12	2.45 ± 0.12 ($P < 0.05$)[b]
C (40% corn oil)	13.7 ± 3.2	3.90 ± 0.86	17.6 ± 3.9	1.39 ± 0.44	2.03 ± 0.66

[a] All values are mean ± SD of 4 serum pools.
[b] Compared to group A.

established only in the lipoprotein value between group C and group A. The large elevation in the serum concentration of LDL_2 in group C was unexpected in view of the constancy of serum cholesterol levels [21] in the three groups. Both the cholesterol and phospholipid in LDL_2 were very significantly elevated in group C as compared to both groups A and B. Further, these values in group B were also significantly higher than in group A. The percent protein (24% of the total lipoprotein) and percent phospholipid (21% of the total lipids) were reasonably constant in the three groups, whereas the percent cholesterol was much greater ($P < 0.05$) in group C (32%) than in other groups (22%).

There was very little contamination in these fractions of VLDL, HDL or albumin (Fig. 1 m to r). The gel patterns obtained using the protein stain confirm the progressive increase in serum LDL_2 starting from group A to group B to group C (Fig. 1, p, q, r). In the gels stained with Amido black, there appeared to be a single prominent band and a faint component immediately ahead of it.

TABLE 3

INFLUENCE OF DIETARY CORN OIL ON RAT SERUM LDL_2 [a]

Group No. (% of fat by wt.)	Total lipid	Protein	Lipoprotein	Cholesterol	Phospholipids
	(mg/100 ml serum)				
A (0% corn oil)	13.4 ± 3.3	4.47 ± 0.44	17.8 ± 3.7	2.98 ± 0.32	2.70 ± 0.47
B (10% corn oil)	19.6 ± 6.4	5.97 ± 0.65 ($P < 0.01$)[b]	25.6 ± 7.0	4.42 ± 0.22 ($P < 0.001$)[b]	3.84 ± 0.57 ($P < 0.025$)[b]
C (40% corn oil)	23.2 ± 1.9 ($P < 0.005$)[b]	6.77 ± 1.2 ($P < 0.025$)[b]	30.0 ± 2.3 ($P < 0.005$)[b]	7.46 ± 0.58 ($P < 0.001$)[b,c]	5.22 ± 0.34 ($P < 0.001$)[b] ($P < 0.01$)[c]

[a] All values are mean ± SD of 4 serum pools.
[b] Compared to group A.
[c] Compared to group B.

...m 3 groups. *a—f*, ...B-prestain, the rest ...*o*, *r*, *u*, *x*, group C.

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TABLE 4

INFLUENCE OF DIETARY CORN OIL ON RAT SERUM HDL_1 [a]

Group No. (% of fat by wt.)	Total lipid	Protein	Lipoprotein	Cholesterol	Phospholipids
	(mg/100 ml serum)				
A (0% corn oil)	10.7 ± 2.6	4.80 ± 0.59	16.1 ± 3.1	2.24 ± 0.64	2.31 ± 0.51
B (10% corn oil)	12.6 ± 3.3	5.30 ± 0.66	17.9 ± 3.9	2.24 ± 0.54	2.43 ± 0.72
C (40% corn oil)	13.4 ± 2.1	5.24 ± 0.20	18.6 ± 2.0	3.37 ± 0.14 ($P < 0.05$)[b] ($P < 0.01$)[c]	2.49 ± 0.35

[a] All values are mean ± SD of 4 serum pools.
[b] Compared to group A.
[c] Compared to group B.

HDL_1 ($1.050 < d < 1.063$)

This lipoprotein is largely responsible for the unique presence of intermediate mobility bands observed during electrophoresis of prestained rat serum [6–8]. Its concentration as well as its lipid composition was virtually unaffected in the three groups (Table 4). One notable exception was the cholesterol content as well as the cholesterol to total lipid ratio in serum HDL_1 of group C which were considerably higher than in the other groups.

In the gel patterns, the principal component was the intermediate band which was approximately midway between the VLDL and albumin components. With both the lipid and protein stains, some contamination with the faster LDL_2 component was apparent (Fig. 1 s to x). Albumin was a trace contaminant in all groups, but VLDL and HDL_2 were consistently absent in all patterns.

HDL_2 ($1.063 < d < 1.125$)

In male Holtzman rats this fraction represents the principal lipoprotein class. Between 0 and 10% corn oil groups, the changes in the lipoprotein concentra-

TABLE 5

INFLUENCE OF DIETARY CORN OIL ON RAT SERUM HDL_2 [a]

Group No. (% of fat by wt.)	Total lipid	Protein	Lipoprotein	Cholesterol	Phospholipids
	(mg/100 ml serum)				
A (0% corn oil)	117 ± 5.1	57.3 ± 0.84	175 ± 4.6	32.7 ± 2.0	32.6 ± 2.3
B (10% corn oil)	118 ± 8.6	59.3 ± 1.2 ($P < 0.05$)[b]	177 ± 8.4	34.5 ± 1.8	32.4 ± 1.1
C (40% corn oil)	107 ± 12	52.0 ± 3.9 ($P < 0.025$)[c]	159 ± 16	31.0 ± 3.3	27.6 ± 2.4 ($P < 0.025$)[b,c]

[a] All values are mean ± SD of 4 serum pools.
[b] Compared to group A.
[c] Compared to group B.

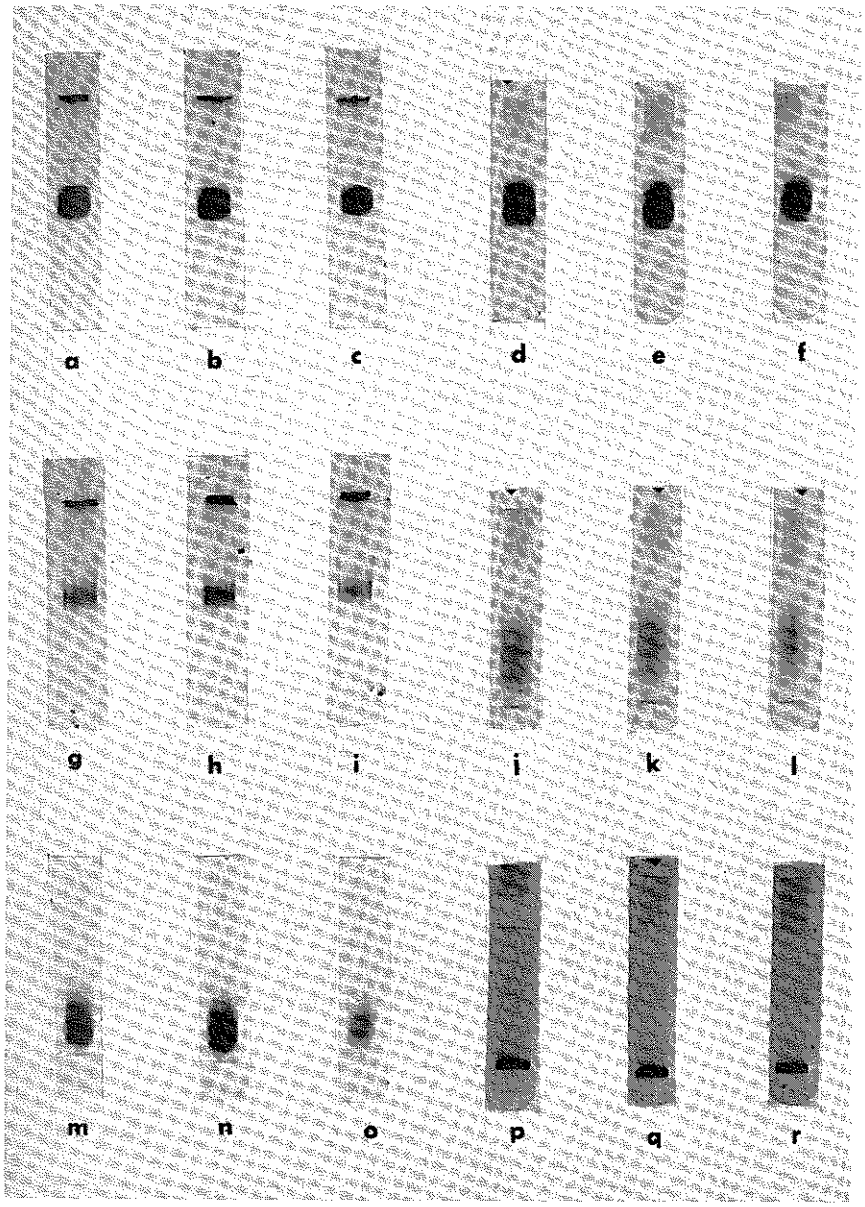

Fig. 2. Representative disc gel electrophoretic patterns of isolated lipoproteins from the 3 groups. *a—f*, HDL_2; *g—l*, HDL_3; *m—o*, HDL_2; *p—r*, HDL_3, impurities (rat serum albumin). *a*, *b*, *c*, *g*, *h*, *i*, Sudan black B-prestain, the rest Amido black stain. *d—f*, tracking dye run to 1.8 cm, *j—r*, 2.8 cm. *a*, *d*, *g*, *j*, *m*, *p*, group A; *b*, *e*, *h*, *k*, *n*, *q*, group B; *c*, *f*, *i*, *l*, *o*, *r*, group C.

tion as well as the lipid composition were rather small (Table 5). The ingestion of a 40% corn oil diet resulted in a decrease in the total lipid, protein, and phospholipid content of this lipoprotein as compared to the other groups. However, only the decrease in the protein and phospholipid moieties of HDL_2

in group C was statistically significant as compared to either group A and/or group B.

With Sudan black B-prestain, the intensity of the HDL_2 component was less in group C as compared to the other groups (Fig. 2 a, b, c). The main component appeared to be composed of two closely migrating bands. A faint non-migrating component (spacer gel—main gel interface) was seen only with the lipid stain.

With the protein stain and a distance of penetration of 1.9 cm, the patterns closely resembled those obtained with the lipid stain (Fig. 2 d, e, f). There were no VLDL or LDL components and contamination with albumin was small. In comparison with groups A and B, the HDL_2 from group C migrated somewhat behind the albumin component. This was more clearly seen when the distance of penetration was set at 2.8 cm. As many as 4—5 bands were seen in this lipoprotein fraction in all groups. Not only were the total HDL_2 band intensities reduced in group C but the band intensities as well as their migration distances were dissimilar when compared with the corresponding HDL_2 patterns in group A and B (Fig. 2 m, n, o). This may indicate possible alterations in apoprotein peptide composition and/or concentration in the HDL_2 fraction from group C.

HDL_3 ($1.125 < d < 1.21$)

As seen from Table 6, it is clear that this high density lipoprotein was not greatly affected by the level of dietary corn oil. As expected, the protein/total lipid ratios were considerably higher in this lipoprotein from all groups as compared to HDL_2. In all groups, the phospholipid/total lipid ratios were surprisingly lower in HDL_3 than in HDL_2. A single unresolved diffuse band was observed in this fraction with the lipid prestain (Fig. 2 g, h, i). A faint non-migrating main-gel component was also present. With Amido black stain, the patterns were similar to those observed with HDL_2 except that the slower components were more prominent (Fig. 2, j, k, l). The slowest component had approximately the same mobility as the principal HDL_1 component which, therefore, makes it difficult to provide a precise band designation for the intermediate mobility lipoprotein components [6,18].

TABLE 6

INFLUENCE OF DIETARY CORN OIL ON RAT SERUM HDL_3[a]

Group No. (% fat by wt.)	Total lipid	Protein	Lipoprotein	Cholesterol	Phospholipids
	(mg/100 ml serum)				
A (0% corn oil)	24.9 ± 7.2	16.6 ± 1.1	41.4 ± 6.6	4.83 ± 0.96	6.28 ± 0.62
B (10% corn oil)	23.9 ± 4.3	16.8 ± 2.1	40.7 ± 4.8	5.28 ± 0.21	5.65 ± 0.6
C (40% corn oil)	22.6 ± 4.5	15.1 ± 0.70	37.7 ± 4.9	5.01 ± 0.35	4.83 ± 0.35 ($P < 0.01$)[b]

[a] All values are mean ± SD of 4 serum pools.
[b] Compared to group A.

TABLE 7

INFLUENCE OF DIETARY CORN OIL ON RAT SERUM BFP[a]

Group No. (% fat by wt.)	Total lipid	Protein	Cholesterol	Phospholipids
	(mg/100 ml serum)			
A (0% corn oil)	47.7 ± 6.3	4340 ± 363	1.15 ± 0.14	24.8 ± 4.1
B (10% corn oil)	60.5 ± 9.1	4408 ± 772	1.18 ± 0.17	22.6 ± 1.0
C (40% corn oil)	38.4 ± 9.7 ($P < 0.025$)[b]	3832 ± 904	1.05 ± 0.10	17.5 ± 6.2

[a] Most values are mean ± SD of 4 serum pools; phospholipid values are mean ± SD of 2 serum pools.
[b] Compared to group B.

Bottom fraction proteins (BFP) and contaminants in crude HDL_3

Mainly for the sake of completeness data have also been provided concerning these fractions (Tables 7 and 8). The dietary treatment did not greatly influence these proteins and their lipid content. Phospholipids were present to a significant extent in BFP. The reason for the large amount of total lipids relative to protein content in HDL_3 impurities is not clear. That this is not an experimental error is indicated by the uniformly high values in all groups as well as the absence of unusually large standard deviations. In view of the relatively low cholesterol and phospholipid content of this fraction, it appears likely that there is a large amount of free fatty acids bound to this protein.

As seen from Fig. 2 p, q, r, there was only one component in the bottom fraction obtained after recentrifuging the crude HDL_3 fraction. The position of this component in the gel coincided with that of rat serum albumin and it did not stain with Sudan black B. When other gel concentrations (5 and 7.5%) were used, this impurity had the same migration distance as rat serum albumin. Although the identity of this protein was not further established by other im-

TABLE 8

INFLUENCE OF DIETARY CORN OIL ON IMPURITIES (ALBUMIN) ASSOCIATED WITH RAT SERUM HDL_3[a]

Group No. (% fat by wt.)	Total lipid	Protein	Cholesterol	Phospholipids
	(mg/100 ml serum)			
A (0% corn oil)	13.8 ± 3.9	42.6 ± 0.73	0.63 ± 0.10	0.85 ± 0.07
B (10% corn oil)	15.7 ± 5.2	44.8 ± 4.2	0.74 ± 0.12	0.51 ± 0.13
C (40% corn oil)	12.4 ± 2.5	44.1 ± 1.4	0.46 ± 0.16 ($P < 0.05$)[c]	1.2 ± 0.03 ($P < 0.025$)[b,c]

[a] Most values are mean ± SD of 4 serum pools; phospholipid values are mean ± SD of 2 serum pools.
[b] Compared to group A.
[c] Compared to group B.

munochemical, chemical or physical measurements, we propose this procedure as a simple way of isolating small quantities of relatively pure rat serum albumin. However, as indicated above, this protein was not free of lipid contamination. The lipid to protein ratio in this fraction far exceeds that of BFP as well as the physiological levels of fatty acids bound to albumin under conditions of maximum mobilization. Whether this enrichment of the albumin is merely due to sequential centrifugation in the presence of a high concentration of salts or to other factors requires further study.

Rat body and tissue weights and diet consumption

The initial average weight of the animals in the three groups was approximately the same (Table 9). Over the four week period, the animals in groups B and C ate less on a weight basis than those in group A. Further, those in group C ate less than the animals in group B. However, when computed on the energy content of the diet, the food consumption was not markedly different in the three groups. Over the entire period, the animals in group A gained slightly less while those in group C gained somewhat more than the rats in group B. The weight differences between groups C and A were always very significant. The rats fed 40% corn oil gained significantly more weight than those fed 10% corn oil at all times except during week 3. The liver weights were approximately the same in all groups, although the livers in group C were slightly, but significantly, heavier than those in group A. The dorsal fat pads weighed about the same in groups A and B but were significantly heavier in group C than in the other groups. While no histopathological examination of tissues was contemplated or conducted, gross macroscopic examination of most organs and tissues failed to reveal any striking deviation from normality. One exception was, however, the appearance of mildly fatty livers in rats fed 40% corn oil.

Discussion

Although many investigators studying the effects of dietary fat have focussed their attention on serum lipids [13,14,23–25], limited data are available in the literature concerning the major density classes of lipoproteins as a result of dietary fat manipulations. For example, Spritz and Mishkel [26] demonstrated that the isocaloric substitution of corn oil for coconut oil in the diet of 12 subjects resulted in decreased levels in the lipid moieties of LDL but not of HDL. In the rat, Reiser et al. [27] have observed that both tallow and safflower oil increased the LDL as compared to a fat free diet. The VLDL concentration was about the same in the fat free and safflower oil groups but was considerably higher in the tallow group. A recent report by Lindall et al. [28] has shown that a coconut oil but not a safflower oil diet in comparison with a low fat control diet elevated the LDL in dogs. The present study is different in that two levels of the same polyunsaturated oil rather than two types of fat have been compared. The large increase in LDL_2 and especially in LDL_2-cholesterol in rats fed a very high level of corn oil as compared to those fed 0 and 10% may cast some doubts concerning the hypothesis of Spritz and Mishkel [26]. They have related the hypocholesterolemic effect of unsaturated fat as compared to saturated fat to differences in their configuration and to the areas occupied

TABLE 9

THE INFLUENCE OF DIETARY CORN OIL ON BODY WEIGHT, FOOD CONSUMPTION, LIVER AND FAT PADS OF RATS[a]

Group. No. (corn oil by wt.)	Body wt. (g)					Average diet consumed during 26 days		Liver wt. (g)	Dorsal fat pad wt. (g)
	Week 0 (N = 28)	Week 1 (N = 28)	Week 2 (N = 24)	Week 3 (N = 21)	Week 4 (N = 16)	g/rat/day (N = 28)	kcal/rat/day (N = 28)	Week 4 (N = 16)	Week 4 (N = 16)
A (0%)	302 ± 11	298 ± 11	329 ± 12	348 ± 13	360 ± 16	20.7 ± 3.0	75.6 ± 11	9.95 ± 0.92	3.53 ± 1.1
B (10%)	301 ± 8.2	308 ± 9.5 $P < 0.05$[b]	338 ± 11 $P < 0.01$[b]	358 ± 11 $P < 0.05$[b]	373 ± 12 $P < 0.05$[b]	19.2 ± 2.0 $P < 0.05$[b]	79.8 ± 8.2	10.3 ± 1.1	4.02 ± 1.1
C (40%)	303 ± 14	322 ± 13 $P < 0.001$[bc]	352 ± 17 $P < 0.001$[b] $P < 0.01$[c]	369 ± 23 $P < 0.01$[b]	389 ± 17 $P < 0.001$[b] $P < 0.01$[c]	15.0 ± 1.1 $P < 0.001$[bc]	84.4 ± 6.5 $P < 0.01$[b] $P < 0.05$[c]	10.6 ± 0.72 $P < 0.05$[b]	6.78 ± 1.3 $P < 0.001$[bc]

[a] All values are mean ± SD of number of rats indicated above.
[b] Compared to group A.
[c] Compared to group B.

by these fatty acids within the lipoprotein particles.

In an earlier study [15], it was shown that while rat serum lipids, especially total cholesterol, were largely unaffected by dietary supplementation of cholesterol, the serum LDL and HDL were drastically altered in opposite directions. In the present study also, the serum cholesterol remained the same [21], in spite of using an excessive level of dietary corn oil. On the other hand, the VLDL decreased substantially while LDL_2 was increased considerably in group C, especially in comparison with group A. Although the total serum cholesterol was unaltered, it was interesting to observe a substantial and very highly significant increase in LDL_2-cholesterol of group C and group B in comparison with groups A and B and group A, respectively. At this time period (week 4) the liver cholesterol in group C was greatly elevated [21]. Recently, Sodhi and Kudchodkar [29] suggested that there were two pools of cholesterol in the human liver, one for anabolic and the other for catabolic purposes. According to their proposal, dietary cholesterol and cholesterol synthesized by the liver would be incorporated into lipoproteins and secreted into blood circulation. During the catabolism of the lipoproteins, the cholesterol moiety enters the catabolic pool and is eliminated as neutral and acidic sterols. On the basis of their hypothesis, it may be speculated that the increase in LDL_2 and in LDL_2-cholesterol in group C may be indicative of a special role for this lipoprotein in cholesterol catabolism.

High carbohydrate diets tend to increase serum VLDL concentration while high saturated fat diets tend to increase LDL concentration in humans [30]. In the present study the concentration of serum VLDL was highest on the fat-free diet and it was lowest in rats given the high-fat diet. The high-fat diet also elevated rat liver triglycerides several fold over that observed in the other groups [21]. It is known that dietary linoleate is preferentially oxidized in the rat and in the human as compared to saturated fatty acids [31,32]. Whether the decrease in serum VLDL observed in rats of group C, concomitant with the rise in liver triglycerides, is due to decreased VLDL synthesis by the liver or due to enhanced utilization by tissues or a combination of both factors is not clear at this time. It is also not known whether high levels of dietary linoleate increase hepatic levels of triglycerides in humans. However, during alchohol intoxication in humans [33] and in certain hyperlipoproteinemic subjects [34], it is known that there is excessive accumulation of neutral fat in the liver. In humans receiving a diet high in linoleate, Nichman et al. [35] have demonstrated a striking decrease in VLDL in hyperlipemic subjects. On the other hand, a concurrent rise in serum LDL was not observed in these subjects, unlike that in rats of group C, perhaps because of the lower level of dietary linoleate used by these workers as well as due to species differences.

It has been recently suggested [4,36] that VLDL is transformed into LDL_2 in two steps by going through an intermediate density lipoprotein (LDL_1). In cholesterol-fed rats, Narayan [15] reported that the isolated low density lipoproteins ($1.006 < d < 1.050$) appeared to be of larger size than in rats on the control diet. Lasser et al. [37] later showed that there was an increase in the concentration of the larger low density lipoproteins, namely LDL_1, in cholesterol-fed rats. However, in the present study (Table 2) the concentration of this lipoprotein (LDL_1) was unaffected in spite of compensatory changes in the

postulated substrate (VLDL) and the presumed end product (LDL_2).

Feeding 1% cholesterol diet to rats resulted in large decreases in serum HDL_1 and HDL_2 [15]. Ingestion of a diet containing 40% corn oil did not reduce rat serum HDL_1 or HDL_3 while it slightly decreased HDL_2. Whereas the increase in LDL was almost as large as observed earlier, the decrease in HDL_2 was only nominal. Whether this was related in some manner to the limited accumulation of cholesterol in the liver of rats in the present study (5.6 mg cholesterol/g liver) as opposed to an enormous deposit in the previous study (45 mg cholesterol/g liver) [15] is not clear at this time.

Recent information concerning the peptides in lipoproteins [1,4] as well as the incorporation of labeled amino acids into lipoprotein peptides [38] have strongly suggested the possibility that VLDL and HDL are the only lipoproteins synthesized by the human and rat liver. The LDL may arise from VLDL by the lipolytic removal of triglycerides since VLDL contains the peptide which is unique to LDL [1]. If, as suggested by several studies [36,38—41], we assume that LDL are not synthesized by the liver, then it would presumably mean that either there was an accelerated conversion of VLDL to LDL resulting from increased utilization of linoleate-rich triglycerides or that there was a decreased catabolism of LDL in rats fed a high level of corn oil. Decreased catabolism of LDL may be ruled out on the basis of a shorter half-life for LDL-cholesterol in safflower oil-fed rats as compared to those on a fat free diet as reported by Reiser *et al.* [27]. On the other hand, there is some evidence [42,43] to indicate the possible contribution of the intestine to the plasma pool of LDL. Furthermore, synthesis experiments conducted with the perfused liver [38], especially using a plasma-free medium, may not be indicative of the existing situation in vivo. Therefore, a third possibility for the increase of LDL_2 in rats of group C is that the peptide (and phospholipid) moieties in LDL_2 may arise largely from VLDL during circulation, whereas a substantial portion of the cholesterol moiety may be added on by the liver (and other tissues to a limited extent) by transfer from the anabolic pool. Undoubtedly, such a transfer will be aided by: (a) a transient increase in apo-LDL peptides in LDL resulting from VLDL degradation; (b) transfer of cholesterol from LDL to other lipoproteins and to tissues; and (c) mainly transfer of-cholesterol of LDL to the catabolic cholesterol pool in the liver. Some support for this proposed mechanism is apparent from differences in the turnover rate of the protein moiety of LDL [44,45] as compared with the turnover rate of the cholesterol moiety of LDL [27]. This view is further strengthened by the recent observation of Sniderman et al. [46] concerning a paradoxical increase in LDL catabolic rate in heptatectomized swine. We suggest that the interaction and exchange of lipids, particularly cholesterol between circulating LDL and the liver may be crucial for its biological stability.

The present study has clearly demonstrated impressive alterations in VLDL, LDL_2-cholesterol as well as in liver lipids as a result of ingestion of a 40% corn oil diet by rats. On the other hand, measurements of routine indicators of nutritional adequacy such as weight gain, food consumption, organ weights and gross pathology failed to signal any deleterious effect of the high corn oil diet. Therefore, the analysis of discrete classes of lipoproteins may prove to be a valuable adjunct in assessing the effect of various levels of different fats. Further-

more, these data emphasize the need for further similar studies in other species including human and nonhuman primates so that a critical reassessment of the effects of polyunsaturated fat can be made [21].

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